Amendments to the Claims

Please amend claims 1 and 22-24. Please cancel claims 13 and 72-77 without prejudice.

Listing of claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

(i) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to a response element, wherein said modified ecdysone receptor comprises:

(a) a ligand binding domain that binds to an ecdysteroid,

(b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and

(c) an activation domain of a transcription factor,

wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

(ii) a DNA construct comprising said exogenous gene under the control of said response element, wherein said response element:

(a) **is a modified response element which comprises, in any order, a first half-site and a second half-site separated by a spacer of 0-5 nucleotides;**

wherein said first half-site has the sequence:

-RGBNNM-,

wherein

each R is independently selected from A or G;

each B is independently selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

each M is independently selected from A or C;

with the proviso that at least 4 nucleotides of each -RGBNNM- group of nucleotides are identical with the nucleotides at comparable positions of the sequence -AGGTCA-;

and wherein said second half-site is obtained from a glucocorticoid receptor subfamily response element ~~**has about 12-20 base pairs,**~~

(b) binds to said modified ecdysone receptor, and

(c) does not bind to farnesoid X receptor (FXR);

said method comprising providing to the cell an effective amount of one or more ligands for said modified ecdysone receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

2. (Cancelled).

~~3.~~ (Previously presented) A method according to claim 1 wherein said modified ecdysone receptor is further characterized as having substantially no constitutive activity in mammalian cells.

~~4.~~ (Previously presented) A method according to claim 1 wherein the DNA-binding domain of said modified ecdysone receptor is derived from a nuclear receptor.

~~5.~~ (Previously presented) A method according to claim 1 wherein said activation domain is obtained from a nuclear receptor.

5. ~~6.~~ (Previously presented) A method according to claim 1 wherein said activation domain is a glucocorticoid receptor activation domain, a VP16 activation domain or a GAL4 activation domain.

6. ~~7.~~ (Previously presented) A method according to claim ~~6~~ 5 wherein said modified ecdysone receptor is VpEcR, VgEcR or GecR.

7. ~~8.~~ (Original) A method according to claim ~~7~~ 6 wherein said modified ecdysone receptor is VgEcR having the amino acid sequence set forth in SEQ ID NO:5.

8. ~~9.~~ (Original) A method according to claim 1 wherein said modified ecdysone receptor is present primarily in the form of a homodimer.

10. (Cancelled).

9. ~~11.~~ (Previously presented) A method according to claim 1, wherein said silent partner is RXR.

10. ~~12.~~ (Previously presented) A method according to claim ~~11~~ 9 wherein said RXR is exogenous to said cell.

13-14. (Cancelled).

11. ~~15.~~ (Original) A method according to claim 1 wherein said ligand is a naturally occurring ecdysone, an ecdysone-analog or an ecdysone mimic.

12. ~~16.~~ (Original) A method according to claim ~~15~~ 11 wherein said naturally occurring ecdysone is α-ecdysone or β-ecdysone.

13. ~~17.~~ (Original) A method according to claim ~~15~~ 11 wherein said ecdysone analog is ponasterone A, ponasterone B, ponasterone C, 26-iodoponasterone A, muristerone A, inokosterone or 26-mesylinokosterone.

14. ~~18.~~ (Original) A method according to claim ~~15~~ 11 wherein said ecdysone mimic is 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, a 1,2-diacyl hydrazine, an N'-substituted-N,N'-disubstituted hydrazine, a dibenzoylalkyl cyanohydrazine, an N-substituted-N-alkyl-N,N-diaroyl hydrazine, an N-substituted-N-acyl-N-alkyl, carbonyl hydrazine or an N-aroyl-N'-alkyl-N'-aroyl hydrazine.

15. ~~19.~~ (Previously presented) A method according to claim 1 wherein said exogenous gene is a wild type gene and/or gene of interest.

16. ~~20.~~ (Previously presented) A method according to claim ~~19~~ 15 wherein said wild type gene encodes products:

the substantial absence of which leads to the occurrence of a non-normal state in said cell; or

a substantial excess of which leads to the occurrence of a non-normal state in said cell.

17. ~~21.~~ (Previously presented) A method according to claim ~~19~~ 15 wherein said gene of interest encodes products:

which are toxic to the cells in which they are expressed; or

which impart a beneficial property to cells in which they are expressed.

18. ~~22.~~ (Currently amended) A method of inducing the expression of an exogenous gene in an isolated cell containing:

(i) DNA encoding a modified ecdysone receptor under the control of an inducible promoter, wherein said modified ecdysone receptor, in the presence of a ligand therefor,

and optionally in the further presence of a silent partner therefor, binds to a response element, and wherein said modified ecdysone receptor comprises:

(a) a ligand binding domain that binds to an ecdysteroid,

(b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and

(c) an activation domain of a transcription factor,

wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

(ii) a DNA construct comprising said exogenous gene under the control of said response element, wherein said response element;

(a) **is a modified response element which comprises, in any order, a first half-site and a second half-site separated by a spacer of 0-5 nucleotides;**

wherein said first half-site has the sequence:

-RGBNNM-,

wherein

each R is independently selected from A or G;

each B is independently selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

each M is independently selected from A or C;

with the proviso that at least 4 nucleotides of each -RGBNNM- group of nucleotides are identical with the nucleotides at comparable positions of the sequence -AGGTCA-;

and wherein said second half-site is obtained from a glucocorticoid receptor subfamily response element ~~**has about 12-20 base pairs,**~~

(b) binds to said modified ecdysone receptor, and

(c) does not bind to farnesoid X receptor (FXR); and

(iii) one or more ligands for said modified ecdysone receptor;

said method comprising subjecting said cell to conditions suitable to induce expression of said modified ecdysone receptor.

19. ~~23.~~ (Currently amended) A method of inducing expression of an exogenous gene in an isolated cell containing a DNA construct containing said exogenous gene under the control of a response element, wherein said response element:

(a) **is a modified response element which comprises, in any order, a first half-site and a second half-site separated by a spacer of 0-5 nucleotides;**

wherein said first half-site has the sequence:

-RGBNNM-,

wherein

each R is independently selected from A or G;

each B is independently selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

each M is independently selected from A or C;

with the proviso that at least 4 nucleotides of each -RGBNNM- group of nucleotides are identical with the nucleotides at comparable positions of the sequence -AGGTCA-;

and wherein said second half-site is obtained from a glucocorticoid receptor subfamily response element ~~**has about 12-20 base pairs**~~,

(b) binds to said modified ecdysone receptor, and

(c) does not bind to farnesoid X receptor (FXR), said method comprising introducing into said cell:

a modified ecdysone receptor, wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation

domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

one or more ligands for said modified ecdysone receptor,

wherein said receptor, in combination with a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said response element, activating transcription therefrom.

20. ~~24.~~ (Currently amended) A method for the expression of a recombinant product detrimental to isolated host cells, said method comprising:

transforming suitable isolated host cells with:

(i) DNA encoding a modified ecdysone receptor, wherein said modified ecdysone receptor comprises:

(a) a ligand binding domain that binds to an ecdysteroid,

(b) a DNA-binding domain obtained from a DNA-binding protein; and

(c) an activation domain of a transcription factor,

wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

(ii) a DNA construct encoding said recombinant product under the control of a response element, wherein said response element;

(a) **is a modified response element which comprises, in any order, a first half-site and a second half-site separated by a spacer of 0-5 nucleotides;**

wherein said first half-site has the sequence:

-RGBNNM-,

wherein

each R is independently selected from A or G;

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each B is independently selected from G, C, or T;

each N is independently selected from A, T, C, or G;

and

each M is independently selected from A or C;

with the proviso that at least 4 nucleotides of each -RGBNNM- group of nucleotides are identical with the nucleotides at comparable positions of the sequence -AGGTCA-;

and wherein said second half-site is obtained from a glucocorticoid receptor subfamily response element ~~has about 12-20 base pairs~~,

(b) binds to said modified ecdysone receptor, and

(c) does not bind to farnesoid X receptor (FXR);

growing said host cells in suitable media; and

inducing expression of said recombinant product by introducing into said host cells one or more ligands for said modified ecdysone receptor, and optionally a silent partner for said modified ecdysone receptor.

25.-38. (Cancelled).

~~39.~~ (Previously presented) A method according to claim ~~15~~, wherein said first half-site is obtained from an ecdysone response element and said second half-site is obtained from a glucocorticoid response element, a mineralocorticoid response element, a progesterone response element or an androgen response element.

~~40.~~ (Previously presented) A method according to claim ~~39~~, wherein said second half-site is obtained from a glucocorticoid response element.

41.-46. (Cancelled).

23. ~~47.~~ (Previously presented) A method according to claim 1, wherein said silent partner is present.

24. ~~48.~~ (Previously presented) A method according to claim ~~47~~ 23 wherein said silent partner is ultraspiracle.

25. ~~49.~~ (Previously presented) A method according to claim 1 wherein said modified ecdysone receptor does not bind to endogenous response elements.

50. (Previously presented) A method for modulating the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

said method comprising providing to the cell an effective amount of one or more ligands for said modified receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

51. (Previously presented) A method according to claim 50, wherein said silent partner is present.

52. (Previously presented) A method according to claim 51, wherein said silent partner is ultraspiracle.

53. (Previously presented) A method according to claim 50, wherein said cell is a mammalian cell.

54. (Previously presented) A method according to claim 51, wherein said silent partner is RXR.

55. (Previously presented) A method according to claim 54, wherein said RXR is exogenous to said cell.

56. (Cancelled).

57. (Previously presented) A method according to claim 50 wherein said modified receptor is further characterized as having substantially no activity in mammalian cells.

58. (Previously presented) A method according to claim 50 wherein the DNA-binding domain of said modified receptor is derived from a nuclear receptor.

59. (Previously presented) A method according to claim 50 wherein said activation domain is derived from a nuclear receptor.

60. (Previously presented) A method according to claim 50 wherein said activation domain is a glucocorticoid receptor activation domain, a VP16 activation domain or a GAL4 activation domain.

61. (Previously presented) A method according to claim 50, wherein said ecdysone response element does not bind to farnesoid X receptor (FXR).

62. (Previously presented) A method according to claim 50 wherein said modified receptor has an altered DNA binding specificity relative to the wildtype receptor from which it is derived.

63. (Previously presented) A method according to claim 50 wherein said modified receptor is present primarily in the form of a homodimer.

64. (Previously presented) A method according to claim 50 wherein said exogenous gene is a wild type gene and/or gene of interest.

65. (Previously presented) A method according to claim 64 wherein said wild type gene encodes products:

the substantial absence of which leads to the occurrence of a non-normal state in said cell; or

a substantial excess of which leads to the occurrence of a non-normal state in said cell.

66. (Previously presented) A method according to claim 64 wherein said gene of interest encodes products:

which are toxic to the cells in which they are expressed; or

which impart a beneficial property to cells in which they are expressed.

67. (Previously presented) A method of inducing the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising an exogenous gene under the control of an ecdysone response element,

(ii) DNA encoding a modified receptor under the control of an inducible promoter, wherein said modified receptor, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor does not bind to

endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

(iii) one or more ligands for said modified receptor;

said method comprising subjecting said cell to conditions suitable to induce expression of said modified receptor.

68. (Previously presented) A method of inducing expression of an exogenous gene in an isolated cell containing a DNA construct containing said exogenous gene under the control of an ecdysone response element, said method comprising introducing into said cell:

a modified receptor, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

one or more ligands for said modified receptor,

wherein said modified receptor, in combination with a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, activating transcription therefrom.

69. (Previously presented) A method for the expression of a recombinant product detrimental to isolated host cells, said method comprising:

transforming suitable isolated host cells with:

(i) a DNA construct encoding said recombinant product under the control of an ecdysone response element, and

(ii) DNA encoding a modified receptor, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

growing said host cells in suitable media; and

inducing expression of said recombinant product by introducing into said host cells one or more ligands for said modified receptor, and optionally a silent partner for said modified receptor.

70. (Previously presented) A method for modulating the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element wherein said modified receptor has substantially no constitutive activity in mammalian cells, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said

DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

said method comprising providing to the cell an effective amount of one or more ligands for said modified receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

71. (Previously presented) A method for modulating the expression of an exogenous gene in an isolated cell containing:

(i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

(ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor has an altered DNA binding specificity relative to the wildtype receptor from which it is derived, and wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said ecdysone response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

said method comprising providing to the cell an effective amount of one or more ligands for said modified ecdysone receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

72.-77. (Cancelled).